A review of sturgeon virosis

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Abstract: Sturgeon, as a kind of rare large-scale economic fish, has high scientific research and commercial value. But virosis was a significant cause of mortality among farm-raised juvenile sturgeon, which brought tremendous loss in the last decades. In this paper, we reviewed the studies on four sturgeon viruses: white sturgeon (*Acipenser transmontanus*) iridovirus (WSIV), white sturgeon herpesvirus-1,2 (WSHV-1,2) and shovelnose sturgeon (*Scaphirhynchus Scaphirhynchus*) iridovirus (SSIV). The content of this review were mainly focused on clinic symptom of infected fish, diagnostic method, virus isolation, prophylactic and remedy. Based on a detailed conclusion and analysis, it is reasonable that molecular biology techniques might be a potential method for sturgeon virosis diagnosis.

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Introduction

Sturgeon, belonging to *Osteichthyes* class, *Actinopterygii* subclass, *Chondrostei* catalogue and *Acipenseriformes* order, is a kind of large-scale economic fish which distributes widely over the north water area of the tropic of Cancer. At present, sturgeons include twenty-six species and subspecies, and have been raised broadly in many countries for its considerable value in economy and ecology.

In China there are 8 species of sturgeon: kaluga (*Huso dauricus*), Sterlet (*A. ruthenus*), Siberian sturgeon (*A. baerii*), Sterlet (*A. ruthenus*), Ship sturgeon (*A. nudiventris*), Chinese sturgeon (*A. sinensis*), Uangtze (*A. dabryanus*) and Chinese paddlefish (*Psephurus gladius*), which distribute in the Yangtze river drainage basin, the Heilongjiang drainage basin and the municipality of Xinjiang Vigur. Amur sturgeon (*A. schrenckii*), Russian sturgeon (*A. gueldenstaedtii*), hybridizing sturgeon, Siberian sturgeon (*A. baerii*), Sterlet (*A. ruthenus*) are some species raised commonly in China in recent decades.

Sometimes, some epizootic diseases with high mortality often lead to significant economic loss to sturgeon fishery. In China, there was an outbreak of disease among cultured sub-yearling sturgeon in 1990s. From 1997 to 1998, the disease leaded to about 210 000 juvenile sturgeon' loss in a breeding farm of Heilongjiang Province. In 2000, the second outbreak of the disease caused more than 200 000 fries to die. Although, the disease has brought tremendous loss to the scientific research and culturing sturgeon fishery, we could not find any lethal bacteria, parasites, fungi from the dead sturgeon. It was suspected that the disease was caused by the viral infection according to its characteristics of epidemiology, dissection and pathology, and the results of experiments of antibiotic inhibition and cure (Zeng 2003).

The study of sturgeon diseases was mainly aimed at sturgeon culturing, but rarely at the wild ones. In the last decades, some foreign virus researchers, especially those in North America, always focused on the common sturgeon (*A. oxyrinchus oxyrinchus*) and white sturgeon (*A. transmontanus*). There were few reports about sturgeon virosis from other species of sturgeon. To our knowledge, only four kinds of viruses were isolated from sturgeon lately, which were white sturgeon iridovirus (WSIV), white sturgeon herpesvirus-1 (WSHV-1), white sturgeon herpesvirus-2 (WSHV-2) and shovelnose sturgeon iridovirus (SSIV).The common etiological characteristics of known sturgeon virosis showed high mortality, fast spread, seasonal and regional outbreak. Especially, virus caused high mortality in juvenile, but could not be isolated from adult sturgeon.

Main sturgeon virosis

White sturgeon iridovirus (WSIV)

The WSIV is an epitheliotropic virus infecting the skin, gills and upper alimentary tract. Illness and death occurs in white sturgeons that are less than 12 months old, and while adults remain unaffected (Disease and Health Related Issues). Up to 95% cumulative mortality has been reported among groups of infected fish in the hatchery and secondary infections with external protozoa or bacteria often contribute to the overall mortality. The source of the virus was assumed to originate from wild sturgeon adults used as broodstocks in captivity, which were collected from the Sacramento River in California, United States of America (Hedrick *et al.* 1990). Infections of the oral mucosa and olfactory organ epithelium are presumed and result in the cessation of feeding, and then progressive emaciation or starvation of the fish, which are the principal external signs of the disease (Watson *et al.* 1998). Now WSIV is the most familiar pathogen to cause the death for the North America cultured white sturgeon and Europe Russian sturgeon (*A. gueldenstaedtii*). Recent evidence also suggests that the disease is present in many regions of white sturgeon habitation (Disease and Health Related Issues).

WSIV was first reported in 1990, which was isolated from North America cultured white sturgeon. Hedrick *et al.* (1992) found that lake sturgeon could be experimentally infected with the WSIV, but the susceptibility of other sturgeon was currently unknown (Hedrick *et al.* 1992). The diagnosis of WSIV is based

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on the observation of pathognomonic infected cells in stained tissue sections collected from infected fish and the isolation of the virus by using sturgeon cell lines. WSS-2 (white sturgeon spleen) and WSSK-1 (white sturgeon skin) are two cell lines commonly used for WSIV isolation. Cells should be grown at 20 ℃ in a temperature-controlled incubator by using standard minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), which can be reduced to 5% after virus inoculation (Diagnostic Manual for Aquatic Animal Disease-2000). Confirmation of WSIV infection relies on neutralization of the isolated virus with polyclonal antibodies. The specific binding of monoclonal antibodies (MAbs) to vital antigens in infected cell cultures or impression smears from infected fish tissues has been investigated as alternatives to cell culture isolation and virus neutralization for identification of WSIV. A set of primers has been developed for the identification of WSIV (Ron Hedrick, personal communication). However, the PCR assay using this primer set is not very sensitive or efficient in amplifying the DNA template of WSIV (Diagnostic Manual for Aquatic Animal Disease-2000). So they wanted to develop another set of WSIV primer for a "nested" PCR assay.

The modes of transmission of WSIV are not completely understood. Horizontal transmission via the water has been demonstrated in the hatchery and experimentally in the laboratory (Hedrick *et al.* 1992). Adults appear refractory to clinical disease and are likely subclinical carriers with intermittent viral shedding (Watson *et al.* 1998). But etiology investigations suggested that WSIV was transmitted vertically from parent to offspring via reproductive fluids or egg content (Bonneville Power Administration FY 2003 Provincial Project Reviews). M. P. Georgiadis *et al.* had also reported that WSIV was probably vertically transmitted from broodfish to their offspring, while tank-to-tank transmission was not a predominant way of spread of WSIV (Georgiadis *et al.* 2001). But the virus has never been isolated or observed in adult fish.

Water temperature had a profound effect on the cause of experimentally – Induced infections of juvenile white sturgeon with the WSIV. Initial *in vitro* investigations indicated that optimum replication of WSIV occurred between 15℃ and 20℃ with poor or no growth outside this range. WSIV-induced mortality ranged from 71% to 54% from the lowest to the highest water temperature tested (10-23℃) (Watson *et al.* 1998).

A new report in August and September of 2001, adduced a diagnosis of white sturgeon iridovirus (WSIV) based on the clinical history and the histologic and ultrastructural features of the affected fish. As the Kootenai River system extends into southwest Alberta, there is a possibility of more widespread infection in wild white sturgeons stocks within Canada than the range of infection currently understood, and efforts to further characterize the distribution of this pathogen are recommended (Raverty *et al.* 2003). We could see that we must do our best to do some efficient work to prevent the WSIV spread worldwide and bring dramatic loss.

Iridovirus was the first virus which caused the sturgeon virosis. There were lots of reports about other fish infected by iridovirus around Chinese water area. A report from Mao *et al.* (1999) on MCP sequences indicated the close relationship of largemouth bass virus to the doctor fish iridovirus. It raised the possibility that viruses from Asia may have been introduced to North America by way of ornamental fish (Mao *et al.* 1999). By analyzing the PCR product sequence results, the infectious spleen and kidney necrosis virus (ISKNV) has 92.5% homologue with red sea bream iridovirus (RSIV), (Deng *et al.* 2000), and the Grouper iridovirus in Taiwan (TGIV) also has high sequence similarity to RSIV. These all suggest the close relation among the various iridoviruses in Asian fish. (Chao *et al.* 2002). So it could be done some sturgeon iridoviruses studies by using other fish iridoviruses diagnose methods.

White sturgeon herpesviruses-1,2 (WSHV-1,2)

In 1991, Hedrick first reported that WSHV was associated with mortality presumed to be the result of the severe infection of the integument and oropharyngeal mucosa, contrast to the infection of epitheliotropic in most other reports. In histology, fish less than 6 months old infected with WSHV-1 have no detectable external disease signs and continue to be fed until death. We could find virions in both the nucleus and cytoplasm of cells directly from infected fish by electron microscopy. It also can be isolated from infected WSSK-1 cells (Hedrick *et al.* 1991). The mature virions had an electron-dense core and a hexagonal nucleocapsid and were surrounded by a coarse electron-dense tegument with a limiting external envelope of 230 nm in diameter. And its nucleocapsid was measured about 110 nm in diameter.

WSHV-2 was first isolated from ovarian fluid of an adult sturgeon and later appeared as a cause of mortality in farmed juvenile white sturgeon (Watson *et al.* 1995). Mortality in adult fish infected with WSHV-2 was generally less than 10%. Experimentally, the shovelnose and pallid sturgeon were susceptible to WSHV-2 but other species were refractive (Mao *et al.* 1999).White sturgeon herpesvirus-2 occurred in older sturgeon and manifests itself as small white blisters which developed into open lesions on the body surface. These lesions were frequently infected with secondary bacteria and/or ectoprotozoa parasites. Internally, the stomach and intestines were filled with fluid but other tissues appear normal. Wild white sturgeon which was infected with WSHV-2 became listless and stopped eating. Current management strategies for controlling WSHV-1 and -2 are avoidance and inspection of potential carrier fish via cell culture assay. It was recommended that sturgeon infected with WSHV-2 could be prophylactically treated with salt and parasiticides to reduce secondary infections in open ulcers.

WSHV-2 can be diagnosed by serum neutralization (Disease and Health Related Issues). And antiserum neutralization can distinguish WSHV-1 from WSHV- 2 (Mao *et al.* 1999).

Shovenose sturgeon iridovirus (SSIV)

In 2001, MacConnell *et al.* reported that they found an iridovirus in cultured pallid (*Scaphirhynchus albus*) and shovelnose sturgeon (*S.* Scaphirhynchus). Clinical signs of WSIV infections in white sturgeon, anorexia and skin lesions, were not typical of pallid or shovenlnose sturgeon infected with this new iridovirus. It is a new viral pathogen, which is similar in appearance to the WSIV, has been identified in Missouri River sturgeon (MacConnell *et al*). So they called it shovenose sturgeon iridovirus (SSIV).

The infected fish were emaciated and had fungal infections in the rostrum and gills. Gill, skin, liver, and kidney tissues were preserved in Davidson's solution, dyed with hematoxylin and eosin, and examined by light microscopic. Microscopic examination of the integument from moribund sturgeon showed the presence of enlarged amphophilic to basophilic staining epithelial cells surrounded by a translucent pericellular space (MacConnell *et al.*2001). Its characteristic features included nuclear

and cytoplasmic enlargement, an eccentric nucleus and intracytoplasmic, refractile, rod-like structures. These cells were strikingly similar to those found in white sturgeon with WSIV infections. Intracytoplasmic fibrillar structures were also seen in cells containing virions. The SSIV shares a very similar morphology to WSIV that possesses a mean diameter of 262 nm with an electron-dense core of 148 nm (Hedrick *et al.* 1992).

Unfortunately, some researchers tried to isolate SSIV in cell culture, such as white sturgeon cell lines or pallid and shovelnose sturgeon cell lines, at several laboratories none of success has been reported (MacConnell *et al.*). It has hindered further characterization of the virus.

Other sturgeon virus disease

White sturgeon adenovirus (WSAV) was found to be associated with infections of the mucosa of the alimengary tract among juveniles reared from 1984 to 1986 (Hedrick *et al.* 1985). But there has not been any report on WSAV since then.

LaPatra *et al.* found that white sturgeon could bring rhabdovirus, infectious hematopoietic necrosis virus (IHNV). But there hadn't any reports on death of infection white sturgeon yet (LaPatra *et al.* 1995).

Conclusions and prospect

In recent decades, sturgeon virosis brought severe loss on sturgeon fishery. By reviewing the studies of sturgeon virosis, we could see the lack of disease information and scientific work. High mortality of sturgeon may be for many reasons, and has a complicated mechanism. By analyzing the disease history of various species sturgeon, bacteria secondary infection was the first reason to the death among most pathogenies (Klinger *et al.* 2000). Transportation, handling, high stocking density, and other stressful conditions may affect the onset and severity of outbreaks to WSIV in white sturgeon in experimental and field settings (LaPatra *et al.* 1994; LaPatra *et al.* 1996).

Control methods currently rely on avoidance of the agent when possible. Because there are currently no methods for detecting the virus in adult broodstocks, quarantine and investigation of juveniles suffering mortality are the principal means to detect WSIV in young fish. Methods to detect the virus in broodstock are currently under development.

The polymerase chain reaction (PCR) is significantly more sensitive than currently used immunological assays such as the enzyme-linked immunosorben tassay (Stoeck *et al.* 1989; Vander *et al.* 1996). PCR has already been used to detect fish-pathogenic virus, epizootic haematopoictic necrosis virus (EHNV) and Bohle iridovirus (Gold *et al.* 1995) which belong to the Iridoviridae, as well as several of the fish viruses such as infectious haematopoietic necrosis virus (IHNV), channel catfish virus (CCV), infectious pancreatic necrosis virus (IPNV) and striped jack nervous necrosis virus (SJNNV), (Arakawa *et al.* 1990; Boyle *et al.* 1991; Lopez-lastra *et al.* 1994; Nishizawa *et al.* 1994). Oshima *et al.* (1996) cloned a small subunit of the ribonucleotide reductase (RNRS) gene of RSIV. Subsequently, PCR successfully amplified virus-specific nucleic acids with a primer set based on the RNRS gene. PCR amplification, which using primer sets based on genomic genes of RSIV, provided a rapid, simple, and sensitive method of diagnosis (Jun Kurita *et al.* 1998). Indeed, iridovirus infections cause devastation epizooeics in fish around the world. We need to do some further work to determine whether the primer sets developed above could amplify other iridovirus.

Currently, cell culture isolation and virus neutralization are still the methods commonly used for identification of the sturgeon viral disease.. However, a highly sensitive method is desirable for detecting the virus in fish at the early stage of the disease. In modern times, diagnostic techniques of molecular biology, especially the polymerase chain reaction (PCR) technology, are being wildly used in disease identification, e.g. a few sturgeon disease researchers have tried to do some work on PCR technique, although not so successful. It is believed that molecular biology method would be the new hotspot to sturgeon virosis.

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